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A TECHNIQUE FOR SETTING AND MOUNTING MICROLEPIDOPTERA

JEAN-FRANÇOIS LANDRY AND BERNARD LANDRY

Agriculture Canada, Centre for Land and Biological Resources Research, Central Experimental Farm, Ottawa, Ontario K1A 0C6, Canada

ABSTRACT. Freshly collected and ammonia-killed microlepidoptera, pinned on minutens, are spread in small, shallow, plastazote-lined boxes with grooves, using either small card points mounted on short pins or translucent setting paper strips to hold the wings. The method produces high-quality specimens, is fast, and uses compact, light-weight, inexpensive equipment. The method is also versatile in that any desired quality of setting, from preliminary, partial setting to the finest setting, can be attained with the same equipment with equal efficiency under any condition, whether at home or on collecting expeditions. The main steps of the method are illustrated. A technique for staging minuten-pinned specimens is also presented.

Additional key words: spreading box, staging, double-mount, ammonia.

During the past 90 years, several papers have presented, with various amounts of detail, techniques for preparing (pinning, setting, and mounting) microlepidoptera (e.g., Kearfott 1904, Calmbach 1921, Lhomme 1926, 1927a, 1927b, Amsel 1935, Holland 1937, Janse 1939, Janmoulle 1943, Charlson 1945, Lindquist 1956, Hodges 1958, Lewis 1965, Tagestad 1974, Zimmerman 1978, Sokoloff 1980, Mikkola 1986). However, our contacts with many lepidopterists indicate that, at least in North America, good and simple techniques for preparing microlepidoptera are not well known. In fact, many North American lepidopterists do not even collect microlepidoptera as routinely as other Lepidoptera, in part because of the perceived inconvenience of preparing them. Microlepidoptera that are collected are often only the larger specimens, in groups such as pyraloids, tortricoids, and large gelechioids.

The paucity of good quality microlepidoptera from North America in many collections is one of the causes for the very slow progress in systematic studies of the Nearctic fauna. Our knowledge of the taxonomy and faunistics of many families of microlepidoptera is shockingly poor. A plea recently has been made for North American lepidopterists to take on the collection and study of microlepidoptera (De Benedictis 1993). Of course the first step in this endeavour is to acquire a *good and efficient* technique for preparing specimens.

There are probably nearly as many ways of preparing microlepidoptera as there are individuals collecting them. The basic method of spreading microlepidoptera is the same as for larger Lepidoptera. How-

ever, some adjustments, both at the time of collecting and of preparation, and in the equipment, are needed because of the small size and fragility of microlepidoptera.

Over the years we have tried every different method and variation of preparation for microlepidoptera that we came to know. While most techniques can yield high-quality specimens, many suffer from being relatively slow or requiring somewhat cumbersome equipment (e.g., spreading boards) ill-suited for prolonged field work under difficult conditions. We sought to develop a technique that offsets these problems, i.e. one that is rapid and usable under any condition with equal efficiency, yet versatile with respect to the quality of preparation desired by the collector. An earlier version of the technique described here was published in French by Landry (1991) but we have modified it slightly, with some additions.

Our method actually combines elements from other methods employed by microlepidopterists, with added refinements. It is based on the concept of setting microlepidoptera on the bottom of a box, which can be traced back at least to Amsel (1935). Modern materials, especially dense polyethylene foam, dramatically enhance the results of Amsel's method. Partial spreading in such boxes is now used by many microlepidopterists on collecting trips (Zimmerman 1978:50–59, Nielsen 1980). The main shortcoming of partial spreading is that special specimens, such as types of new species or those needed for photography, may need subsequent relaxation for final spreading. The technique exposed here offers the possibility of a full range of quality of preparations, from unspread to fully spread with as much care as a perfectionist may wish, all with the same equipment and with hardly any extra time. The technique may be used in the field, in the lab, or at home. The necessary equipment is very compact, light-weight, inexpensive, and easily made. We have tested the method with tens of thousands of microlepidoptera over the past few years, under conditions varying from local day trips to month-long expeditions in the tropics (including camping).

In addition to the actual technique of setting microlepidoptera, we offer some suggestions for handling specimens when they are collected in the field, and for staging (double-mounting) spread specimens. Appropriate handling of collected microlepidoptera is as critical as the actual setting in obtaining high quality specimens, and so is the final staging to insure safe preservation in subsequent handling.

COLLECTING

The facility and rapidity of the technique outlined here rests on working with the freshest specimens possible. Moths are placed indi-

vidually in glass vials upon collecting and kept *alive* until the time of pinning and setting. Upon returning from the field, vials are stored in a cool, dark place if the specimens cannot be prepared immediately. The ideal place is the refrigerator, or a cooler box if one is on a prolonged field trip. We have been able to keep specimens alive for up to five days in this manner, although we recommend delaying as little as possible (some moths will begin to show some wear even after 1–2 days in the refrigerator). Refrigeration is particularly useful if one has had a large catch on one day and there is not enough time to prepare all specimens immediately after they have been collected. We recommend preparing the smallest microlepidoptera as soon as possible, as they will die more quickly from dehydration. Once dead, small moths tend to dry very quickly and become difficult to relax and spread. In the humid tropics, small microlepidoptera will dehydrate quickly inside vials (often in just a few hours) and are best set as soon as possible. Always begin by preparing the smallest specimens first, working up to larger ones. Refrigeration, even if available, should probably not be used for tropical microlepidoptera from those regions that seldom experience temperatures below 10°C, because the relative cold will kill many of them.

Vials. Collecting vials should preferably be made of glass and close with an easily removable stopper that can be opened with a single hand (the other may be busy holding a net). We use glass vials that are 65 mm long and 19 mm in diameter, closed with a rubber stopper. Stoppers should be as little wedge-shaped as possible, otherwise smaller microlepidoptera will crawl in the space between the stopper and the vial neck and damage themselves. We carry about 100 vials for most day-time collecting, at least twice as many for night-time collecting at a light. Experience will dictate the adequate supply. During day-time collecting, care must be taken that the vials are not exposed directly to or heated by sunlight, otherwise the moths will quickly die and dry. If possible, avoid plastic vials (snap-cap type), especially with the smaller specimens, because the static charge that such vials accumulate through handling and friction will damage the squamous cover of the moths and increase the rate of wear.

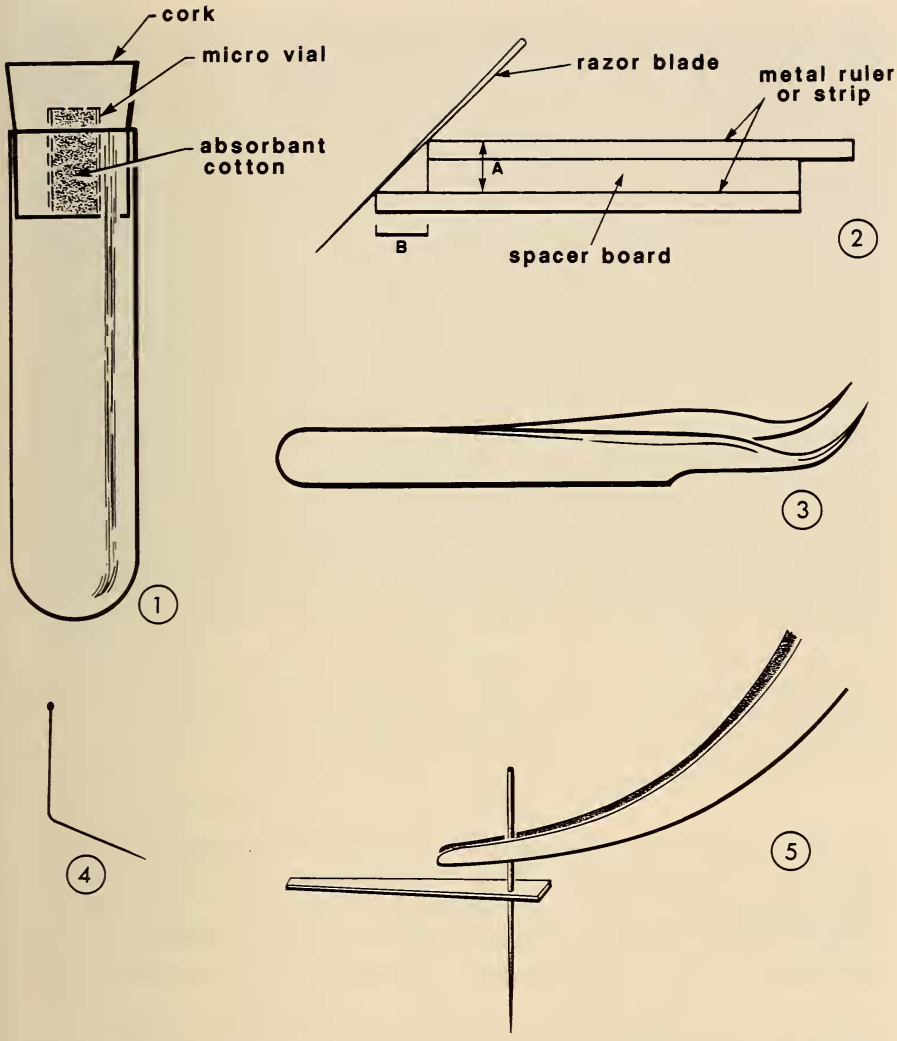
A word of caution is necessary if one is setting reared specimens: *never* set a freshly emerged moth. Allow at least 24 hours (longer if a genitalia dissection may be required) for the moth to harden sufficiently. Without this precaution, wings may curl, crumple, or droop after removal from the setting box, and if the genitalia are later dissected, structures will be insufficiently sclerotized and difficult to prepare adequately.

POISON AND KILLING TUBES

The choice of poison is, of course, a matter of personal preference, availability, etc. We strongly recommend ammonia (ammonium hydroxide): it has a quick knock-down action and leaves freshly killed specimens beautifully relaxed and ready to be spread immediately with the greatest ease. We have tried other killing agents and methods, but ammonia is the one that has given us the best results. The ammonium hydroxide solution should be as concentrated as possible. A laboratory-grade solution containing about 30% ammonia and 70% water is preferable because it has a very fast knock-down action. Household ammonia, generally a murky liquid, is weaker and unsuitable.

For killing tubes, we use glass tubes closed with cork stoppers into which a small microvial is inserted, loosely stuffed with cotton (Fig. 1). Five to ten minutes before using a tube, the cotton is imbibed with a few drops of ammonia solution, and the tube closed to let the ammonia concentration rise. This type of killing tube offers nothing inside against which struggling moths may rub; the disadvantage is that the tubes need to be recharged more frequently, approximately once every 2–3 hours of continuous use (when opened several times periodically). When setting large numbers of microlepidoptera, we use up to 10 tubes at a time to minimize recharging, and place only 2–3 moths per tube at a time. It is essential to check for and wipe traces of moisture or sweating on the walls of the killing tubes. Charged tubes may be laid on their side to prevent any ammonia from possibly running down the sides, although this will not be a problem if a modest quantity is used. When tubes are not in use for more than a day or so, it is preferable to leave them open and remove the cotton swab from the stopper to allow then to dry thoroughly.

Ammonia has a few disadvantages: it tends to sweat in a tube if an excessive quantity is used or if it is too warm (tubes must not be exposed to heat or direct sunlight)—but this is a disadvantage common to most liquid poisons; it loses strength relatively rapidly in a frequently open tube; and fumes are choking, irritating. Weak ammonia must *not* be used for moths with green, red, or orange pigments because the long exposure needed to kill them may cause discoloration. If the ammonia is strong though, this is not a problem providing that the moths are removed as soon as they are dead. In case of doubt about possible discoloration, one should use another poison, preferably ethyl acetate (subsequent relaxation may be necessary). With strong, concentrated ammonia, we have not had discoloration problems. Generally we have found that the advantages of ammonia far outweighed its disadvantages, none of which presented a real problem if it was used with the pre-



FIGS. 1–5. Materials required for preparing microlepidoptera. **1,** Killing tube; **2,** superimposed, offset rulers to cut symmetrical V-shaped grooves, A = B for 45° grooves; **3,** curved forceps used to handle minutens; **4,** bent standard pin used to assist pinning and spreading; **5,** card triangle mounted on shortened pin used to hold set wing in the point method.

cautions outlined above, and that it was no more inconvenient to use than any other poison.

Recently we have experimented with a solid form of ammonia, ammonium carbonate, a salt with the appearance of cyanide crystals. Upon

contact with the ambient humidity, the crystals decompose into gaseous ammonia, carbon dioxide and water vapor (Gilligan and Gilligan 1990). Killing tubes are made simply by packing a 1–2 cm thick layer of crystals in the bottom and covering them with a smooth, porous material [e.g. artificial foam sponge (Gilligan and Gilligan 1990)]. We used plastic caps (from snap-cap vials) punctured with many minute pin holes to cover the crystals. Plaster cannot be used because the water it contains will instantly dissolve all the crystals and produce all the ammonia at once. We obtained satisfactory results with ammonium carbonate if used for small numbers of specimens. Disadvantages are that the rapidity of killing decreases markedly compared to liquid ammonia if one opens the tubes frequently; also if there are too many specimens in a tube and it is warm, the moisture content may rise to the point where, upon cooling, crystals may form on the specimens; such crystals are then very difficult to remove. For these reasons we find ammonium carbonate less satisfactory than ammonium hydroxide.

Ethyl acetate also works well but we found that it has a tendency to stiffen many microlepidoptera if they are left in the killing tube a few minutes too long; hence, some relaxation is sometimes necessary. Like ammonia, it is volatile, and tubes need frequent recharging and may "sweat" if heated. It is also flammable and will dissolve some plastics. Generally, we have found ethyl acetate to be less satisfactory than ammonia in quickly producing ready-to-spread specimens.

KILLING

Remove the cork, insert one moth, close the cork. Repeat with other tubes. When there is one moth in each tube, start again with the first tube, ensuring that the moth is stunned. Continue until there are 2–3 moths per tube. Stunning takes less than five seconds when the ammonia is strong but may stretch to 10–15 seconds after tubes have been opened several times. Moths should be left in the killing tubes for at least 15 minutes to ensure they are dead. Very small moths (Nepticulidae, small Gracillariidae, for example) can be removed sooner. A time saving strategy in the subsequent setting operations is to segregate specimens by size at the killing stage. This way, at the setting stage, one does not have to switch back and forth among various spreading boxes with different groove widths.

SETTING EQUIPMENT

Spreading boxes (Figs. 6, 17–18). We use shallow, clear polystyrene plastic boxes; currently we have two sizes, 11cm × 11cm × 2cm, and

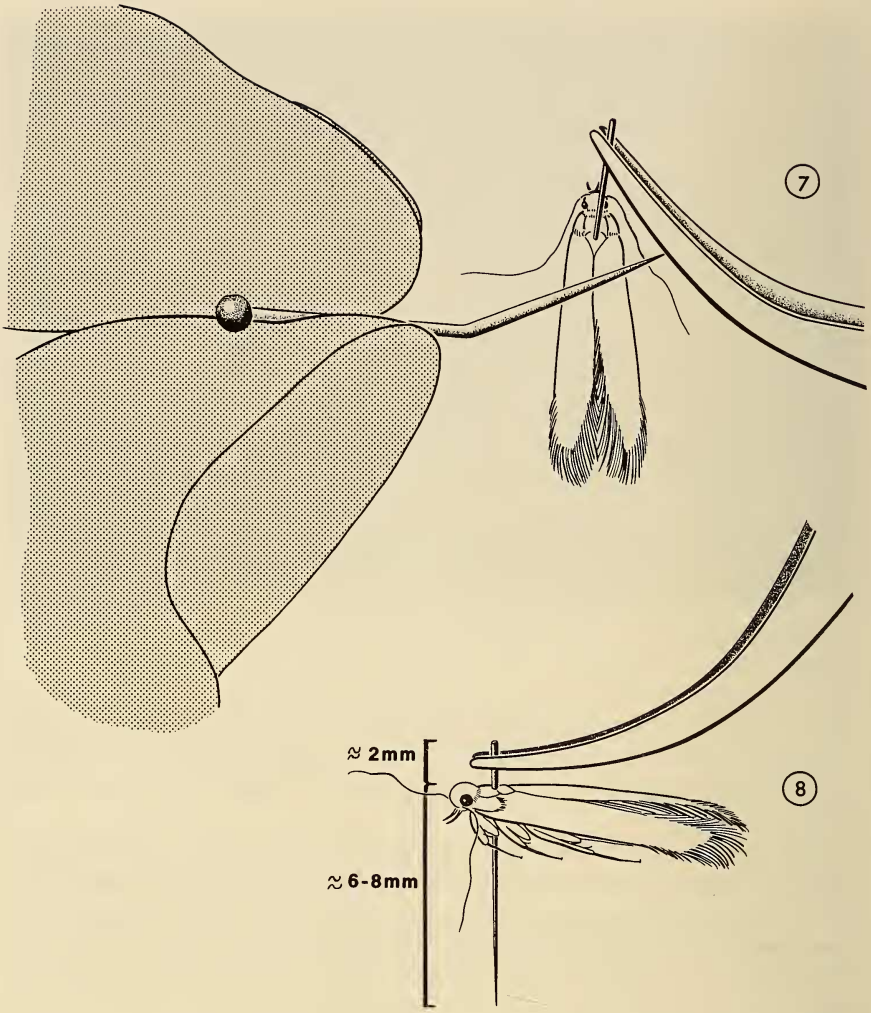
FIG. 6. Spreading box. The actual lid is used as bottom on which the plastazote is glued. Scale in cm.

12cm × 8cm × 2cm, obtained from different suppliers. Actual dimensions are not important, as long as boxes are relatively small, preferably shallow (for compactness), with a low-edge lid, rigid, and relatively airtight (or pest-proof).

For a spreading surface we use Plastazote®, a dense, smooth polyethylene foam. We found this material best because it affords the following advantages: the surface acquires a small static charge through handling, which helps wings cling slightly and facilitates spreading; it grips the pins firmly and leaves no pin holes; it sustains hardly any wear.

A 1-cm thick piece of plastazote is glued inside the lid of a spreading box (we use all-purpose, non-toxic white glue). Gluing the foam inside the lid (using the bottom as lid) eliminates edges to the spreading surface, greatly facilitates work of the hands, and maximizes use of the spreading surface.

Before gluing the foam into the boxes, we cut three or four V-shaped grooves with a razor blade. To obtain grooves with perfectly symmetrical sides, we use two metal rulers or strips, with one being taped on top of the other and propped up by a thin board; the edge of the top ruler is offset from the edge of the lower one by a distance equal to that of the ruler + board thickness (Fig. 2). To cut, the blade is slanted



FIGS. 7–8. **7,** Inserting the minuten while holding the body with a bent standard pin; **8,** Minuten-pinned specimen, showing approximate height on 1-cm long minuten.

and abutts both edges. Symmetrical grooves facilitate spreading. We use a series of spreading boxes with various groove widths, these varying from 1–5 mm (2mm and 3mm are the most frequently used widths). It is not necessary to have square grooves with vertical sides, as on standard spreading boards. In fact, the sides of V-shaped grooves often provide direct support for the abdomen.

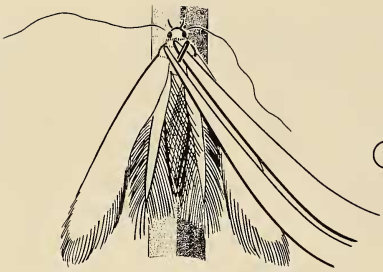
Minuten pins. Use of minuten pins involves subsequent staging or

double-mounting, so this is distasteful to many a lepidopterist. Whatever the perceived difficulty, inconvenience, or time factor involved, we emphasize that this is by far the best and safest way of obtaining fine-quality microlepidoptera. Double-mounted specimens can sustain rougher handling without damage and are far less likely to lose their abdomen, a very frequent problem with microlepidoptera that are mounted on fine standard pins (00 or 000), which are very springy. The genitalia are critical for the specific determination of numerous species of microlepidoptera, hence the abdomen must not be lost.

There are different qualities of minutens available on the market. For the best results, and to avoid frustration, one should use the best quality stainless steel minutens. Avoid black-enameled minutens, which have a tendency to rust (guaranteed if one is in the tropics) and have tips that more easily "hook" (being made of softer metal). The difference in price between stainless steel and black-enameled minutens is small. Diameters of the most useful sizes are 0.20 mm, 0.15 mm, and more rarely 0.10 mm (for nepticulids and other tiny microlepidoptera); some British brands label their minutens A1 (0.14 mm) and B1 (0.19 mm) (1 referring to the shortest length, usually 10–12 mm).

Most minutens are excessively long and must be shortened down to no more than about 1 cm for the larger ones (0.20 mm) or 6–7 mm for the finer ones (0.15 mm and 0.10 mm). If minutens are not shortened, the excess length jutting either above or below the specimens will greatly increase the risk of breakage or damage during handling of the double-mounts (fingers pinching the minuten while grasping the stage-supporting pin will spring the specimen and likely send parts flying, most commonly the weakly-attached, all-precious abdomen). A rapid method of shortening a large number of minutens is to cut narrow strips of plastazote (often the latter's thickness is conveniently 1 cm or 7 mm), to insert minutens all the way through the strips (ensuring that their tips do not extrude), and trimming off the excess length close to the strip surface with good scissors or pin cutters. To maximize efficiency later in the setting process, we prepare large quantities of trimmed minutens in advance. Minuten-loaded plastazote strips can be packed side by side in an insect mounting tray or small shallow cardboard box. A protective layer of plastazote is glued on the bottom of the tray or box. Strips are then laid upright, side by side, and held in place with pins inserted through the sides of the tray or box; any remaining space can be filled with plastazote. Use a box narrow enough for the holding pins to pierce through at least half of the strips from one side.

Tools. We use curved forceps for handling minuten pins (Fig. 3). The inner surface of the grasping end must be smooth (not striate). While fine straight forceps could be used, we found curved forceps to



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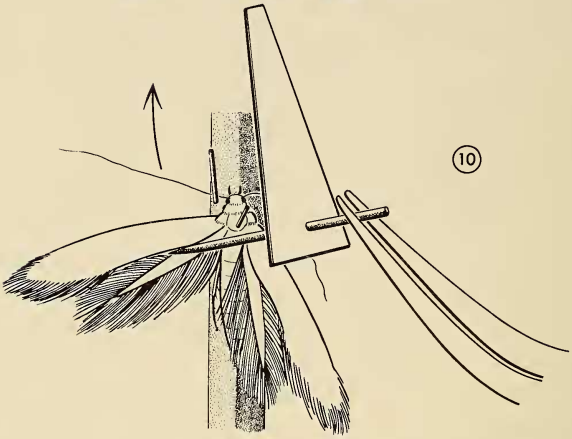

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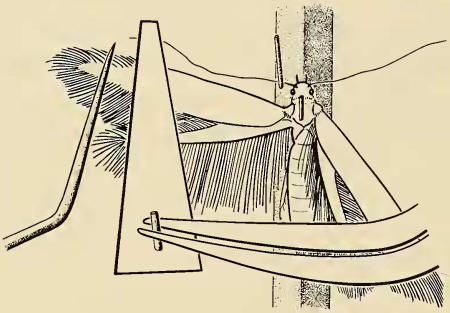

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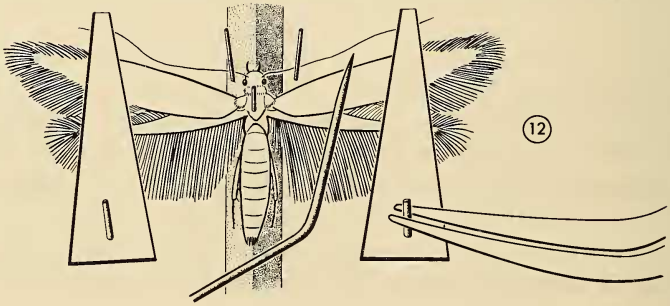

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be a much more versatile tool for the task. A large standard pin (e.g. no. 4) bent at an obtuse angle (Fig. 4) provides an inexpensive tool, instead of a second pair of forceps, to help in holding the specimens or the wings during pinning and setting. It is important to use a pin that is not too fine because the point may catch and rip into the wings too easily.

Setting triangles (Fig. 5). Triangles are used to hold the wings in place once they are spread. They are made with a point punch (of the type commonly used for mounting small insects) from moderately thick, very smooth or glossy card and inserted no more than half way up on short pins. We use two sizes of triangles [7 mm long with pointed end (Fig. 17) and 10 mm long with truncate end (Figs. 10–12)] for different sizes of microlepidoptera. We mount them on pins no. 00 cut down to 1 cm in length (trimmed the same way as the minutens). Do not use minutens for mounting triangles because they are too fine to insert easily into the relatively thick card stock of the triangles. When mounting triangles, check that the side with rough edges (produced by the punch on the underside of the paper) is turned upwards (check with a magnifying lens if necessary). If this simple precaution is not taken, much damage to the wing scales will occur because of the rough edges of the triangles. As for minutens, a large supply of mounted triangles should be readied, pinned in shallow boxes. Triangles are re-used indefinitely or until they become loose on the supporting pins.

Pinning pad. White cotton fabric folded several times into a pad about 1 cm thick and 10 cm × 10 cm makes an ideal surface to pin microlepidoptera. The fabric must be as soft as possible. This surface prevents specimen compression when pinning and the fabric fibres tend to hook the moth claws, thus reducing slippage. Avoid paper towels of any kind, they are usually too rough. The thickness of the pad must be greater than the length of the minutens so that the pad does not have to be lifted up when pushing the minutens through the specimens.

Humid container. This serves to hold pinned specimens to prevent them from drying while they await setting. If this precaution is not taken, the smaller microlepidoptera will begin to dry in a mere few

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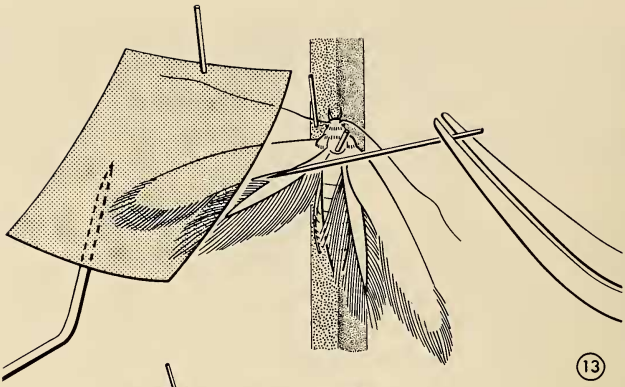
FIGS. 9–12. Setting with the point method. **9,** Inserting the specimen into the groove with the wings partly opened; **10,** Moving one set of wings forward with the point-holding pin; note the antenna held in position with a minuten; **11,** Setting the wing into position with the mounted point while holding it with the bent pin; **12,** Repeating the operation with the other side.



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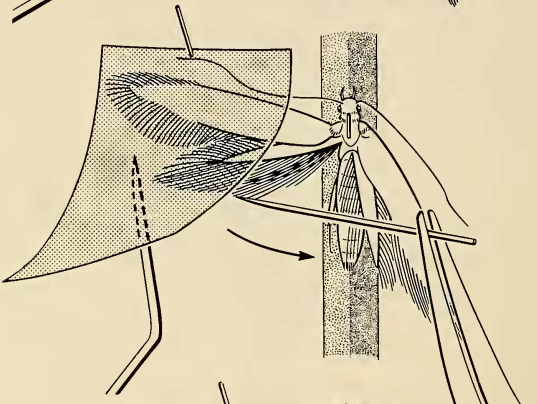

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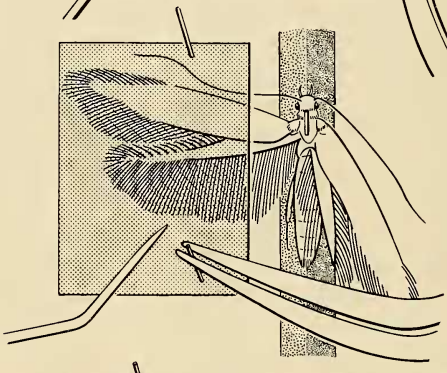

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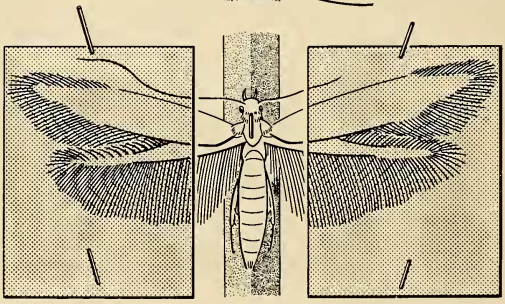

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minutes in dry air and become difficult to spread by the time one gets to the last few of a batch. The container is simply made from a plastic petri dish or similar small plastic dish or box with a loosely fitting lid. The bottom of the dish is lined with wetted tissue or filter paper. A small plastazote pad serves to hold specimens. In very dry conditions, the inside of the lid may be *lightly* misted to increase ambient moisture in the container (too much moisture could drip on the specimens).

PINNING AND SETTING

For best results (and less eye strain) the pinning and setting operations should be done with magnifying lenses or under a low-power stereoscope (up to about 5×).

Pour the freshly killed moths on the cotton pad and pin them. Insert the minuten through the center of the mesothorax (mesoscutum) or at the suture between the mesoscutum and mesoscutellum (the mesoscutellum is the roughly triangular or diamond-shaped area behind the center of the mesothorax). Try to keep the pin in line with the center of the thorax, otherwise the wing muscles may become transfixed, which renders spreading more difficult. To ensure that a specimen is squarely pinned, apply very slight pressure on its dorsum with the tip of the bent pin (or another curved forceps) to prevent the body from rolling sideways while the minuten is inserted into the mesothorax (Fig. 7). The minuten must be inserted far down so as to leave no more than about 2 mm protruding above the moth, enough to manipulate it comfortably with forceps (Fig. 8). Of course, the height of specimens with unusual structural modifications such as long palpi recurved over the body or thoracic crests should be adjusted appropriately in order to leave sufficient minuten length for the forceps; such specimens may require longer (untrimmed) minutens.

Place pinned specimens in the humid container. Prior to this, if one wishes, the wings may be partly opened by gently blowing on them from behind the moth with a slight puff of breath. Before proceeding with setting, another series of specimens is transferred to the killing tubes. Hence, there will be specimens ready for pinning when the first batch has been set. We usually proceed in batches of no more than 15–25 moths.

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FIGS. 13–16. Setting with the paper method. **13,** Moving one set of wings forward with a minuten while lifting the paper strip with the bent pin; note the minuten holding the antenna; **14,** Combing the fringe; arrow indicates direction of combing movement; the combing minuten touches the tip of the fringe lightly; **15,** Pinning the paper strip down to secure the wing into position; the minuten holding the antenna may be removed as it is usually no longer necessary; **16,** Set specimen.

Take specimens out of the humid container singly for setting. If the wings are still closed, gently blow on them from behind, then insert the specimen into the groove (Fig. 9). Lift the wings and partly push them forward with the tip of the closed curved forceps inserted beneath the wings. Tuck the legs into the groove. With a minuten position the antennae so that they form a widely obtuse V, holding them temporarily by placing minutens behind their base. If the fringes are matted, lift the wings a little and comb the fringes by brushing them with the tip of the triangle's pin in a movement going from the apex of the wings toward the body.

To fix the wings into position, we use two different procedures.

(1) Points method (Figs. 10–12). This method may be a bit faster than the paper method (see below). Although excellent, it sometimes gives slightly inferior results, and makes it more difficult to set the antennae properly.

Using a mounted triangle, bring one pair of wings forward by pushing on the hind margin of the forewing with the tip of the pin. Usually, if this movement is delicately executed, both wings will move together because of the coupling. Do not pierce the wings. While holding the wings into position with a slight pressure of the bent pin held in the other hand, put the triangle on top of the wings as close to the apex of the hindwing as possible and push it down sufficiently to immobilise the wings. The triangle must lie flat against the wing surface and must not be pressed down too strongly or it will leave a mark. It may be necessary to adjust the position of the hindwing slightly, which sometimes will be a little too far back or too far forward. One or more triangles may be added to better hold the wings of larger or broad-winged microlepidoptera or to prevent them from curling up.

Repeat the procedure for the other side. To prevent set specimens from hindering hand work over the spreading box surface, it is best to proceed in transverse rows instead of filling one groove after another.

For someone having difficulty using both hands simultaneously, the following variation may be applied: using a mounted triangle as outlined above, move a pair of wings only halfway forward then insert the triangle over the wings just sufficiently to prevent the wings from slipping back but ensuring that they can still be moved; with a minuten move the wings into their final position (the wings should stay in place) and with the forceps maintained closed, gently push down the top of the pin holding the triangle until the wings are flat. Positioning of the wings in this way may have to be done in several stages for some specimens. The other hand may hold the spreading box. With this variation, one can proceed by filling one groove after another if desired.

(2) Paper method (Figs. 13–16). The second procedure uses small

strips of thin, translucent setting paper and is essentially similar to the standard technique used to spread larger Lepidoptera on a normal setting board. The paper strips are held down with 0.20 minutens. This technique can yield the finest specimens because the entire surface of the wings is held flat, and the antennae can be set properly with ease. It is a little more cumbersome and may take a little more time depending on individual ability. In our own experience, however, it takes about the same amount of time as the triangle technique, if one has prepared and has ready the necessary materials, such as pre-cut pieces of setting paper and minutens.

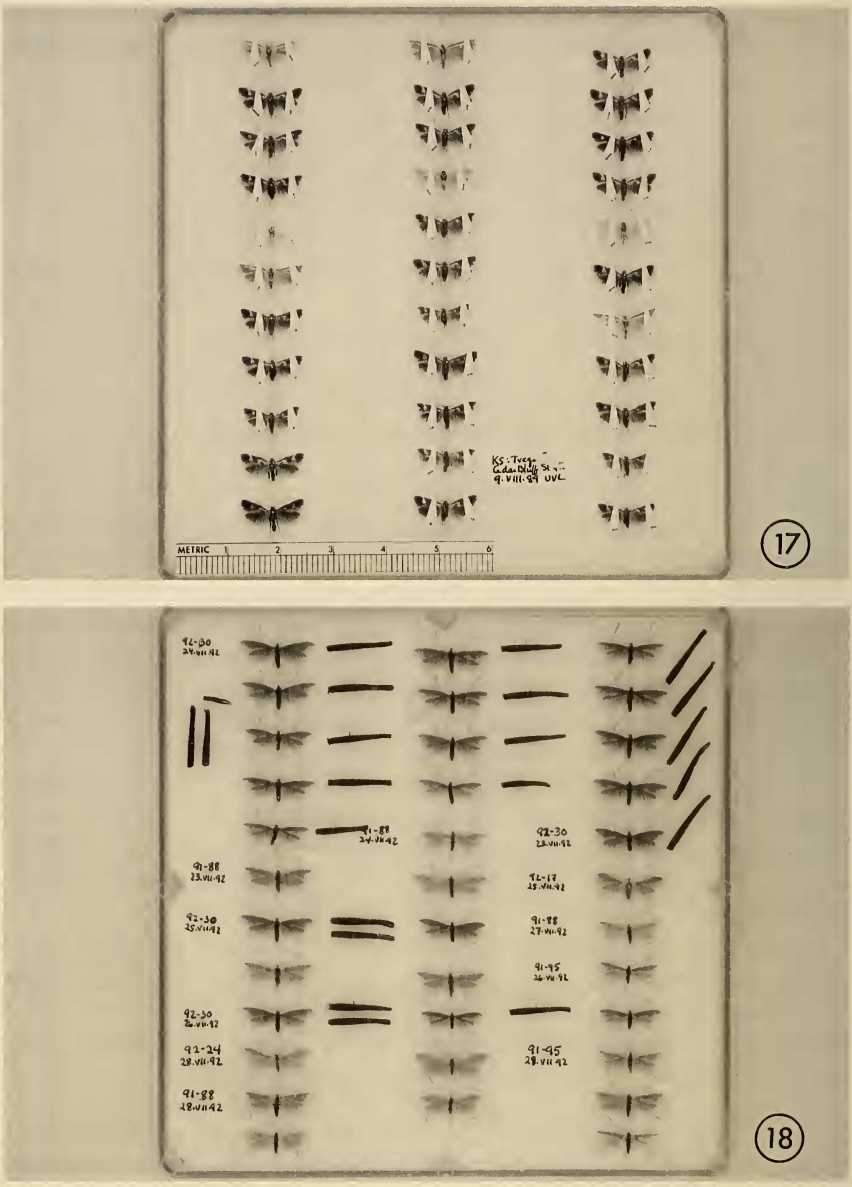
Cut many small pieces of setting paper, just long enough to cover the antennae and one set of wings, prior to spreading. For most microlepidoptera, the strips we use are about 1–1.5 cm long and about 5 mm wide. Four minutens are usually needed to spread one moth. For increased speed, sets of four paper-holding minutens may be pinned beside each groove of an entire row before the setting begins. When one row has been filled with specimens, another series of minutens is placed along the next row, and so on.

After pinning the moth and having set the antennae as described above, pick up a paper strip by stabbing it with a minuten and pin it just ahead of the antenna to cover the half-opened wings. Check that the curvature of the paper faces upward. With the bent no. 4 pin (or another pair of curved forceps) held in one hand, slightly lift the posterior end of the paper from beneath. With another minuten held with curved forceps in the other hand and working from behind the hind margin of the forewing, push the wings into position. When both wings are positioned, drop the paper strip, hold it down with the tip of the bent no. 4 and pin it behind the hindwing with a second minuten. Repeat on the other side.

Choosing the appropriate groove width will facilitate spreading. A groove too narrow will force the legs up and put pressure on the thorax, thus hindering wing movement. A groove too wide will result in either the specimen swinging on the pin when the wings are pushed on one side, or in an insufficient portion of the wing surface resting flat.

Before placing the specimen into the groove, the plastazote surface may be gently rubbed with the tip of the closed forceps to create a charge of static electricity which will help in spreading the wings. This is not necessary, however, if one is using the paper strip method, and it is not recommended with very small microlepidoptera such as nepticulids because the charge will be too strong and may push the wings up vertically.

With a fresh, fully relaxed moth and some practice, the whole operation of pinning and setting takes no more 30–60 seconds. With



FIGS. 17–18. Examples of filled spreading boxes. **17,** Point-set specimens; **18,** Paper-set specimens (larval cases beside reared specimens). Scale in cm.

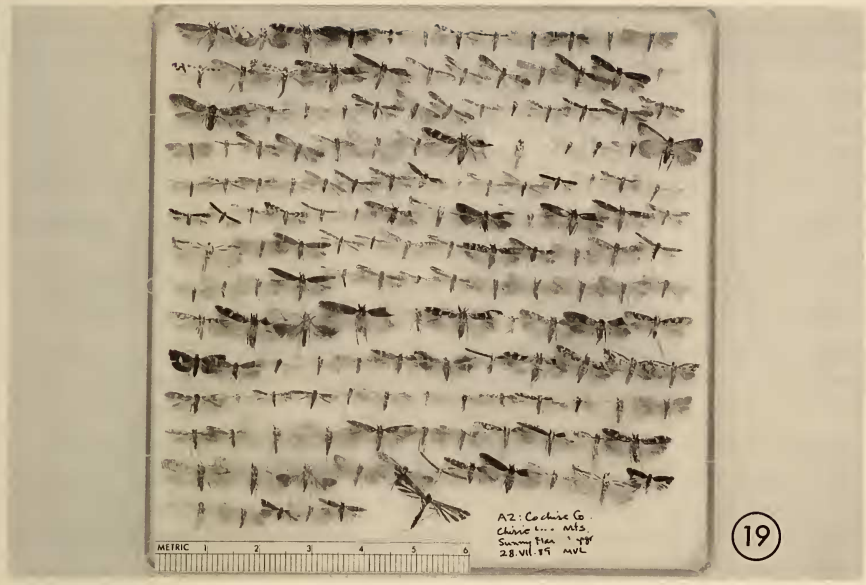


FIG. 19. Example of packed spreading box in which previously set and dry specimens are overlapped like shingles to conserve space. This 11 × 11 cm box contains 166 specimens.

practice, specimens can be set quite closely behind one another into the grooves to conserve space (Figs. 17–18).

Stunning prior to spreading is sometimes used, instead of killing, when time is short (Sokoloff 1980). If specimens are only anesthetized (stunned) prior to spreading, it is necessary to pin a small cotton swab imbibed with ammonia into the spreading box and close it for about 15–20 minutes to kill the moths. If the spreading box is made of polystyrene-base plastic, avoid ethyl acetate because it will dissolve the plastic. We do not use the stunning method because we find it inconvenient, especially in the field.

Label the specimens as usual and leave them in the spreading boxes in a dry place for at least two weeks, or preferably for as long as possible. If one does not provide enough time for the specimens to dry, the tips of some wings may curl up or droop. In humid regions, it is advisable to secure a few crystals of 4-chloro-m-cresol in the boxes to prevent molding. Once the moths are dry, full boxes should be sealed tightly with tape until ready for staging.

When in the field for an extended time and spreading boxes are in short supply, or to reduce the number of boxes being transported, space can be saved by removing specimens from the grooves after drying

and packing them somewhat like shingles (Fig. 19). Specimens are pinned slanted in transverse rows, with the left wings of a specimen partly overlapping the right wings of the preceding one. This allows for large quantities of specimens to be stored in little space. An entire collection of several thousands of microlepidoptera can be carried in this way in a handbag on a plane instead of being placed in regular baggage, thus maximizing the safety of specimens that may represent months of field work in a remote region.

Some authors have recommended heat-drying because, supposedly, moths that have been heat-dried will never have drooped wings (Amsel 1935). This is, however, a delicate and risky operation that must be done very carefully with *very low* heat (ca. no more than 40° C). We have tried drying on a few occasions and are rather weary of it. We have noticed that several microlepidoptera tend to become a little greasy when dried with heat (noticeable under magnification). Another problem is that the plastazote of the spreading boxes may warp slightly from being heated. We think that it is preferable to see some wing drooping occur later in the collection than risk damaging specimens in heat-drying. Wing drooping will be minimized or virtually eliminated if specimens are allowed to remain set in the spreading boxes for an extended period.

STAGING

To be placed in collections, dry minuten-pinned microlepidoptera must be mounted individually on small rectangular blocks, which are inserted on standard (# 3 or 4) insect pins. This is referred to as staging or double-mounting. Specimens should always be mounted *singly* on a block, complete with all necessary labels on the supporting pin, except perhaps in cases of mated pairs which may be staged together. It is very annoying to find two or more microlepidoptera belonging to different but superficially similar species that have been staged together with a single label; such specimens have to be remounted separately and new labels produced. Multiple mounts also increase the risk of misassociation of subsequently made genitalia slides.

Staging blocks. It is more efficient to prepare large quantities of blocks in advance. Traditionally, blocks have been cut from strips of polypore fungi (especially from birch bracket fungus). Normally it is easy to procure polypore strips from naturalist supply houses, but periodically they tend to become very difficult to obtain.

Plastazote provides a superior substitute. It is comparatively inexpensive, available in practically infinite supply, extremely regular in density, practically unalterable, and pest proof (we once had a supply of polypore strips heavily infested with ciid beetles). Plastazote allows

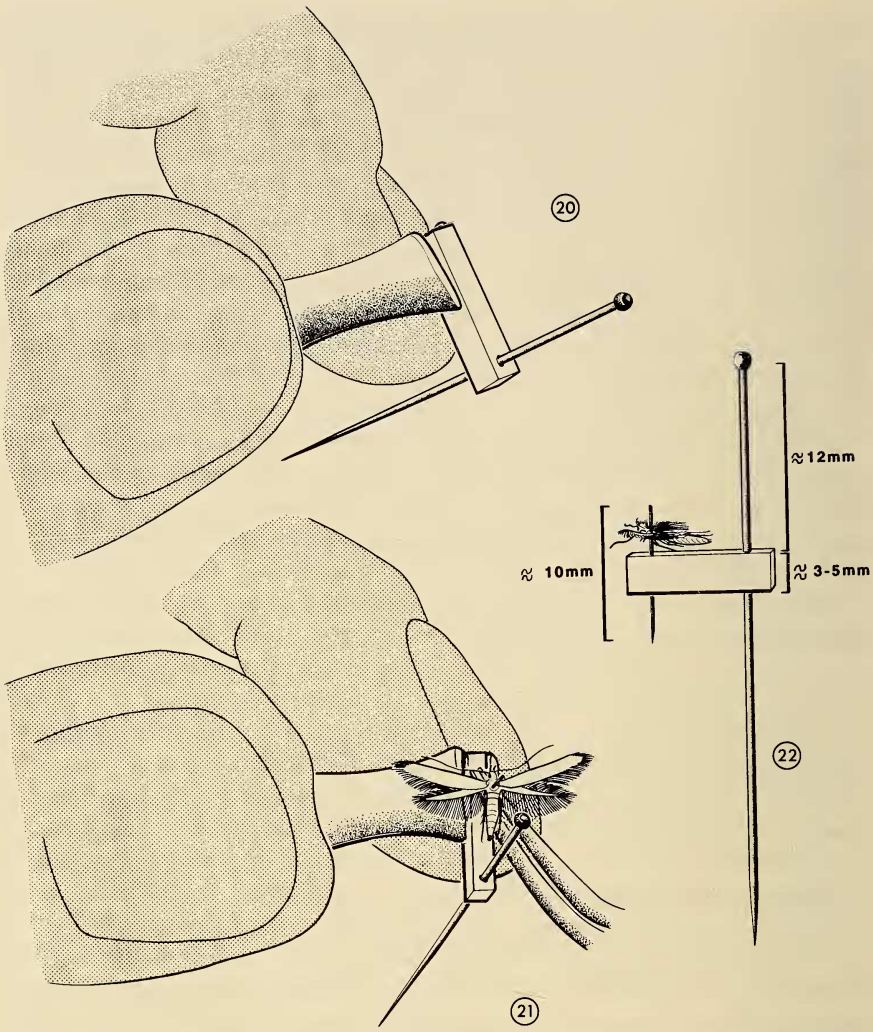
the finest minutens to be inserted without effort and provides remarkable protection from shocks and vibrations. Other materials such as balsa, cork, and polystyrene-based foam ("styrofoam") should be avoided because they are either too hard to insert the minuten without risking damage or are not rubbery enough to hold firmly the pin and the minuten (the latter is a problem of balsa and polystyrene-based foams, on which minutens frequently become loose). Blocks made of a silicon rubber compound are used by some but their durability is uncertain in insect drawers where they may be affected by fumigants; we have seen a set of such blocks that were about 15 years old and that exuded a greasy substance which seeped up the minutens and coated the specimens. It is also harder to insert a minuten into silicon rubber, which is a springy material.

The length of the blocks varies with the size of the specimens. Ideally, we think that they should be about as long as the length of the moth from its head to the tip of its abdomen plus about 3 mm to provide space for the legs and the supporting pin. The width and height vary little and are from 2–3 mm (width) and from 2–4 mm (height). We recommend the use of as few sizes of blocks as necessary to maintain some uniformity to the collection. A cutting board with preset guides and mounted razor blade can be made to speed the cutting of large numbers of uniformly sized blocks. It is essential to mount the blocks on standard pins *prior* to double-mounting the moths. Staging blocks must be inserted up to a height that will leave adequate clearance between the specimen and the head of the supporting pin to allow for safe handling of the whole mount (Fig. 22); we recommend at least a 1 cm clearance.

Staging procedure (Figs. 20–21). To facilitate staging, use one pair of forceps with curved tips and another with broad, flattened tips.

With the flat-tip forceps, hold the pinned block in front of you. With the curved forceps, take the specimen by holding the minuten from beneath the specimen and insert slightly into the block. Check that the plane of the wings is perpendicular with the axis of the pin and adjust the inclination if necessary. Still grasping the minuten from beneath the specimen, pull it down into the block to the point where the venter of the moth is about 1 mm from the surface of the block.

Holding the minuten from beneath the specimen for insertion is especially critical if one is using polypore blocks. Polypore blocks vary markedly in hardness and pushing the minuten down while grasping it from above the specimen may cause the minuten to bend or spring, usually resulting in damage to the moth. Using plastazote blocks generally obviates this danger but grasping the minuten below the moth reduces the risk of damage in case of slippage of the forceps.



FIGS. 20–22. Staging or double-mounting. **20,** Holding with flat-tipped forceps a staging block mounted on a standard pin; **21,** Inserting the specimen on the stage, clasping the minuten from below the specimen; **22,** Staged specimen, showing good heights for safe subsequent handling.

It is important to insert the minuten as far down as possible, while not touching the stage, in order to secure the specimen (Fig. 22). Specimens protruding high on the block risk getting damaged in subsequent handling as much as those with overly long minutens jutting high above the body.

Final Remarks

The techniques described above may seem laborious, but what takes many words to explain is actually executed in just a few seconds. With some practice, one can easily pin and set up to 30–40 microlepidoptera of fine quality per hour.

If there is no time or desire to fully spread all the moths that are collected, one may at least spread the wings partly and brush the fringes. Provisional spreading (Amsel 1935, Zimmerman 1978: pp. 48-ff, Nielsen 1980, Mikkola 1986), with subsequent relaxation and spreading if necessary or desired, is a good compromise where time is short such as during expeditions aiming at sampling as many specimens as possible. Damaged or rubbed specimens that may be worth collecting for some reason may be partially spread to save time.

Generally we do not use light traps and prefer to collect microlepidoptera at light on a sheet. Although light traps afford several advantages in sampling and are often necessary for surveys, we find that one is easily overwhelmed by the abundance of specimens so obtained, that a significant amount of time is necessary to sort the microlepidoptera from other Lepidoptera and insects, and that most specimens sustain a certain amount of rubbing and damage. If there is no time to relax and set trap-collected specimens right away, they should be placed on slightly damp cotton in tight containers and kept in a freezer.

Methods that involve killing the specimens immediately upon capturing them (as in light traps) and storing them for an indeterminate period of time (e.g. by freezing), generally necessitate some period of relaxation in a humid chamber before proper setting can be performed. Such specimens are usually not quite as easy to spread as freshly killed specimens and are not ideal for the point-setting technique described above, although satisfactory results can be obtained with adequate relaxation and using the paper-strip technique. Specimens that have dried unspread usually cannot be subsequently relaxed and spread. Some lepidopterists who have tried our technique complained that it was not quite as easy as we told them but, when pressed for details of how they proceeded, most conceded that they had killed their specimens upon collecting and spread them a little later without relaxation. We reiterate that working from fresh, live specimens killed just before setting is central to the ease and rapidity with which microlepidoptera can be set with the technique described here, and to obtaining high-quality specimens. Of course, some experience is necessary to achieve the best results; one is unlikely to obtain perfect microlepidoptera after attempting to set only a dozen specimens.

It is a truism that fine, well-prepared specimens are easier to identify.

This is particularly true for microlepidoptera, whose small size puts them at a disadvantage over the larger Lepidoptera when it comes to studying them (incidentally, lepidopterists facing space limitations to house their collection of macros should seriously consider taking up the collection of microlepidoptera!). Many well prepared microlepidoptera can be recognized at a glance. On the other hand, rubbed, damaged, or badly mounted specimens may be quite difficult to recognize, even to family, particularly if they are unspread.

Unavoidably, processing microlepidoptera soon after their collecting will take more time and seem more laborious than for larger Lepidoptera that are simply papered or pinned for subsequent setting. It can be argued, however, that the time involved strictly in spreading microlepidoptera is no more than for spreading macros; in fact spreading microlepidoptera is faster. The main difference is that one should do it right at the time of collecting for best results. The resulting quality of the specimens makes it well worth the effort.

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